variability. In contrast, a single embryonic stem cell (ESC) line can be repetitively cryopreserved, thawed, expanded, and differentiated into various cellular components serving as a renewable and well-characterized stem cell source. We, therefore, determined whether ESCs could be used to reconstitute marrow and blood in major histocompatibility (MHC) mismatched mice. To induce differentiation toward HSC *in vitro*, ESCs were cultured in methylcellulose with hematopoietic cytokines, stem cell factor (SCF), interleukin-3 (IL-3), interleukin-6 (IL-6). After 7-10 of culture, H2b ESC-derived cytokine induced hematopoietic stem cells (c-kit$^{+/}$CD34^{+}) were isolated by flow cytometry and injected either intra bone marrow (IBM) or intravenously (IV) into lethally irradiate MHC mismatched H2^{d} recipient mice. From two to twenty weeks after injection, the peripheral blood demonstrated increasing donor derived H2^{b} positive mononuclear cells that included donor derived T lymphocytes, B lymphocytes, monocytes and granulocytes without clinical or histologic evidence of graft versus host disease (GvHD). Mixed lymphocyte culture (MLC) proliferation assays demonstrated T cell tolerance to both recipient and donor but intact 3rd party proliferative responses and interferon-γ production. Embryonic stem cells may be used as a renewable alternate marrow donor source that reconstitutes hematopoiesis with intact immune responsiveness without graft versus host disease despite crossing MHC barriers.

IMMUNE RECONSTITUTION

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ANTI-3RD PARTY VETO CTLS DEPLETED OF HOST REACTIVE CLONES RETAIN A BROAD TCR REPERTOIRE: A POTENTIAL NEW SOURCE FOR ADOPTIVE IMMUNE THERAPY IN BONE MARROW TRANSPLANTATION

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CD8+ cytotoxic T lymphocytes (CTLs) are endowed with a powerful capacity to induce specific tolerance against their histocompatibility antigens (veto). Very recently, we developed a new approach to deplete CTLs of host-reactive clones by stimulating the donor T cells against 3rd party stimulators in the absence of exogenous IL-2. It could be anticipated that this procedure, which leads to a marked frequency reduction of anti-host clones, should also be associated with substantial narrowing of the TCR repertoire, thereby limiting the use of these veto cells for tolerance induction. In the present study we used a new technique (TcLandscape) which enables qualitative and quantitative analysis of Vβ chain usage at the CDR3 length distribution level to compare the repertoire of anti-3rd party CTLs to that of the original donor. Mouse and human anti-3rd party CTLs were prepared as previously described. Mouse splenocytes of Balb/c and FVB were used as effectors and stimulators, respectively. Human PBMC from normal donors were stimulated against EBV transformed cell lines, completely mismatched for HLA class1. cDNA samples obtained from mouse and human CD8+ responder T cells before (day 0) and after initiation of culture (days 22 and 33 respectively) were analyzed for Vβ repertoire. The analysis showed that in CTLs of both human and mice, a significant amplification or reduction was evident only in particular CDR3 lengths of specific Vβ families. Thus, mouse anti-3rd party CTLs exhibit marked clonal reductions in Vβ3, Vβ5, Vβ12, Vβ13 and Vβ16. Changes in the latter one were most dramatic and it is almost oligoclonal. In contrast, only minor alterations were found in all the other Vβ families indicating a preservation of broad repertoire. Analysis of CDR3 lengths of anti-3rd party human CTLs showed similar preservation of broad repertoire along with marked reduction of particular CDR3 length clones in Vβ12, Vβ15 and Vβ21. These findings are of particular relevance to BMT, as it might enable to use anti-3rd party CTLs not only for enhancement of engraftment, but also for adoptive transfer of immunity during the early post transplant period. Furthermore, these results raise new possibilities for molecular probing of the antigens which trigger alloreactivity in mouse and in man.

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ADOPTIVE TRANSFER OF EX VIVO COSTIMULATED AUTOLOGOUS T-CELLS AFTER AUTOTRANSPLANTATION FOR MYELOMA (MM)ACCELERATES POST-TRANSPLANT T-CELL RECOVERY

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Ex-vivo co-stimulation of autologous T-cells using anti-CD3/anti-CD28-conjugated magnetic beads may restore T-cell responsiveness toward MM cells. 40 patients (pts) received ex-vivo co-stimulated autologous T-cells after autotransplantation. The mean age was 58 (range 43-72), 72% were male, 24% had IgA paraproteins, 12% had del 13 or complex karyotypes and the median β2m level at diagnosis was 3.32 mg/L (range 1.09-73.7). Lymphocyte collections were followed by cyclophosphamide (4.5 g/m^2) G-CSF for stem cell mobilization melphalan (200 mg/m^2 or 140 mg/m^2 for pts ≥70). T-cells were cultured for 12 days with anti-CD3/anti-CD28-immobilized immunomagnetic beads & IL-2 supplementation (100 units/ml). During a run-in phase, 12 pts received T-cells post-transplant (~day +12) alone; afterwards, 28 pts participated in a 2 × 2 randomization in which they received T-cells "early" (day +12) or "late" (day +100) after transplant also received 2 immunizations with the pneumococcal conjugate vaccine (PCV, Prevnar®) days +30 +90 or 3 immunizations (prior to T-cell collection, days +30, +90) to test immune responses. 27 pts received 1 or more PCV immunizations and there were no grade 3/4 adverse events. Anti-pneumococcal antibody T-cell response assays are underway for pts who completed the study. 33 pts received a mean of 8.11 × 10^9 costimulated T-cells (range 1.6-11) all infusions were well tolerated. There were no delayed adverse effects except for grade 1-2 facial/upper body rashes in 6 pts (median 13 days after T-cells) and 1 episode of grade 2 conjunctivitis. At T-cell harvesting, the mean % of CD3 + cells in culture was 94.2% the mean T-cell doubling level was 5.2. Among the randomized pts, at day + 42 post-transplant (~30 days after T-cell infusion for the "early" groups), the mean CD4/CD3 count was 679/μl (95% CI, 347-1012) for the "early" T-cell recipients vs 278/μl (95% CI, 59-497) for the "late" T-cell recipients (T-cells not yet infused) [P = 0.03]. The mean CD8/CD3 counts were 1826/μl (95% CI, 1275-2376) 1105/μl (95% CI, 404-1806) for the "early" and "late" T-cell recipient respectively at day +42 [P = 0.07]. 32 pts were evaluable for clinical responses (8 are too early). There were 6 CRs, 15 VGPRs (≥90% reduction in paraprotein levels), 10 PRs (50-90% reductions), 1 pt had no response. Post-transplant infusions of ex-vivo expanded autologous T-cells are feasible and well-tolerated may be associated with accelerated T-cell recovery.

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ACCELERATED IMMUNE RECONSTITUTION USING LLME TREATED DONOR LYMPHOCYTE INFUSIONS

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Delayed immune reconstitution is a major cause of morbidity and mortality after T-cell depleted allogeneic progenitor cell transplant (PCT). To accelerate immune reconstitution without GVHD, we have administered escalating doses of L-leucyl-L-leucine methyl ester (LLME) treated lymphocytes (DLI) to 9 patients post CD34+ cell-enriched PCT in an ongoing phase I trial. LLME's cellular toxicity occurs after its polymerization by dipeptidyl peptidase, leading to selective depletion of cells with cytotoxic effector